JPP
Journal of
Pharmacy and Pharmacology

JPP 2007, 59: 1463–1471
© 2007 The Authors
Received March 27, 2007
Accepted July 31, 2007
DOI 10.1211/jpp.59.11.0002
ISSN 0022-3573

Prediction of human pharmacokinetics – renal metabolic and excretion clearance

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Abstract

The kidneys have the capability to both excrete and metabolise drugs. An understanding of mechanisms that determine these processes is required for the prediction of pharmacokinetics, exposures, doses and interactions of candidate drugs. This is particularly important for compounds predicted to have low or negligible non-renal clearance (CL). Clinically significant interactions in drug transport occur mostly in the kidneys. The main objective was to evaluate methods for prediction of excretion and metabolic renal CL (CL_R) in humans. CL_R is difficult to predict because of the involvement of bi-directional passive and active tubular transport, differences in uptake capacity, pH and residence time on luminal and blood sides of tubular cells, and limited knowledge about regional tubular residence time, permeability (P_e) and metabolic capacity. Allometry provides poor predictions of excretion CL_R because of species differences in unbound fraction, urine pH and active transport. The correlation between fraction excreted unchanged in urine (f_e) in humans and animals is also poor, except for compounds with high passive P_e (extensive/complete tubular reabsorption; zero/negligible f_e) and/or high non-renal CL. Physiologically based in-vitro/in-vivo methods could potentially be useful for predicting CL_R. Filtration could easily be predicted. Prediction of tubular secretion CL requires an in-vitro transport model and establishment of an in-vitro/in-vivo relationship, and does not appear to have been attempted. The relationship between passive P_e and tubular fraction reabsorbed (f_{reabs}) for compounds with and without apparent secretion has recently been established and useful equations and limits for prediction were developed. The suggestion that reabsorption has a lipophilicity cut-off does not seem to hold. Instead, compounds with passive P_e that is less than or equal to that of atenolol are expected to have negligible passive f_{reabs}. Compounds with passive P_e that is equal to or higher than that of carbamazepine are expected to have complete f_{reabs}. For compounds with intermediate P_e the relationship is irregular and f_{reabs} is difficult to predict. Tubular cells are comparably impermeable (for passive diffusion), and show regional differences in enzymatic and transporter activities. This limits the usefulness of microsome data and makes microsome-based predictions of metabolic CL_R questionable. Renal concentrations and activities of CYP450s are comparably low, suggesting that CYP450 substrates have negligible metabolic CL_R. The metabolic CL_R of high-P_e UDP-glucuronyltransferase substrates could contribute to the total CL.

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Acknowledgements: I thank my colleagues at AstraZeneca R&D Södertälje for inspiration and for reviewing the manuscript.

Note: This paper includes personal opinions of the author, which do not necessarily represent the views or policies of AstraZeneca.

Introduction

The kidneys have the ability to both excrete and metabolise drugs, and are one of the major organs involved in drug elimination (Hall & Rowland 1983; Rowland & Tozer 1995; Masereeuw & Russel 2001; Walton et al 2004; Shitara et al 2005). Methods for prediction of renal clearance (CL_R) in humans and an understanding of the mechanisms that determine this process are needed in order to predict plasma concentration–time profiles and potential for interaction, and thus in the selection of appropriate candidate drugs (CDs). This is particularly important for compounds that have, or are predicted to have, low or negligible non-renal CL. The likely implication of striving to find and develop CDs with good metabolic stability (for example, with the aim to enable once-daily dosing) is that these compounds will be excreted mainly via urine and/or the bile. Elimination via these routes is difficult to predict and these pathways have high potential for drug–drug interactions. Ho and Kim (2005) listed 26 clinically important drug transport interactions, most of which appear to relate to renal and bile excretion (decreased excretion; increased systemic exposure) of actively secreted compounds with limited passive permeability (P_e).

Allometry and physiologically based in-vitro/in-vivo (PB IVIV) methods are commonly used to predict human pharmacokinetic (PK) parameters. Allometry is a poor predictor of CL and volume of distribution (V_D). For CL, simple allometry has an average overprediction potential: more than 2-fold error for about one-third of predictions, and a range of 140-fold underprediction to 5800-fold overprediction, with it undesirable exposure profiles and safety risks (Fagerholm 2007a). For V_D, maximum prediction errors are large (>10-fold), and greater than 2-fold prediction errors are found for 35% of substances (Fagerholm 2007b). Reasons for poor prediction include species differences in binding and metabolic activities. Differences in binding to blood components are also likely to influence allometric predictions of CL_R. PB IVIV methodology, which has a more sound rationale, enables more accurate and precise predictions of hepatic CL (CL_H) and V_D to be reached (Fagerholm 2007a, b). For most of the PB IVIV predictions, errors are more or less negligible (<25%) (Björkman et al 2001; Shibata et al 2000, 2002; Björkman 2004; Fagerholm 2007a, b). PB IVIV predictions of CL_H predictions based on intrinsic CL_H determined using microsomes are poorer (considerable underprediction) than those obtained using hepatocytes (Iwatsubo et al 1997; Ito et al 1998; Obach 1999; Naritomi et al 2001; Ito & Houston 2005; Fagerholm 2007a). Poor predictions from microsomes probably relate to permeation (there is no an outer cell membrane to permeate), binding (some binding sites are absent) and low/absent metabolic activity (microsomes contain only membrane-bound enzymes and no cytosolic enzymes, and they seem to have reduced capacity to metabolise the most unstable compounds) (Lavé et al 1999a; Obach 2001; Masimirembwa et al 2003; Fagerholm 2007a, c). These features also indicate that tubular cell microsome data probably have limited applicability for prediction of CL_R.

In order to achieve good predictions of CL_H from human microsome and hepatocyte data, empirical scaling factors are used (Fagerholm 2007a, c). Without these, the CL_H will be underpredicted by 4–9-fold on average (Fagerholm 2007a). One implication of neglecting to use such scaling factors is that we may be misled into believing that stability will not be a problem and that excretion will be the major route of elimination.

The main objective of this paper was to evaluate methods for prediction of excretion and metabolic CL_R in humans. Determinants of CL_R were also evaluated.

Evaluation of determinants for renal clearance

About 125 mL plasma water (~10% of renal blood flow rate (Q_R), which is about 1200 mL min^{-1}) undergoes ultrafiltration in the glomeruli each minute (Leggett & Williams 1995; Rowland & Tozer 1995; Walton et al 2004). Unbound compounds with a molecular weight (MW) up to about 2000 g mol^{-1} can pass through this sieve (Rowland & Tozer 1995). Thus, the glomerular filtration CL (CL_{filtr}) for most drugs and CDs equals the glomerular filtration rate (GFR; 125 mL min^{-1}) multiplied by the unbound fraction (f_u) in plasma ($f_{u,pl}$).

Passive and active tubular transport from the blood to the lumen is determined by the interplay between basolateral (blood side) P_e and the surface area of the tubular cells, f_u in blood ($f_{u,bl}$), characteristics of blood flow (Q_R, time available for tubular cell uptake, convection/mixing), and the architecture of the tubular system. Available data indicate that tubular cells have relatively low passive P_e, and that the basolateral membrane has lower passive P_e than the brush-border membrane on the luminal side (Masereeuw & Russel 2001). Madin–Darby canine kidney (MDCK) cells, which are commonly used for P_e screening, have a passive P_e similar to that of the Caco-2 cell line (Fagerholm et al 1999; Irvine et al 1999; Lundquist et al 2002). This indicates low passive tubular excretion and re-absorption capacities, and that permeation could be the limiting factor for tubular metabolism of compounds that are not highly permeable. Uptake into tubular cells from the blood is limited by a short residence time. The ratio of renal blood volume (100 mL; Leggett & Williams 1995) and Q_R gives a tubular transit time of about 5 s. According to Rowland & Tozer (1989), the blood residence time at the proximal tubular secretory site is considerable longer: about 30 s.

The proximal tubuli are the primary site of carrier-mediated transport from blood to tubular fluid and urine (Masereeuw & Russel 2001; Shitara et al 2005). Secretion capacity shows remarkable heterogeneity along the proximal tubuli (Masereeuw & Russel 2001), which is one of the factors that makes it difficult to predict renal elimination of actively secreted compounds. The basolateral tubular cell membrane not only differs from the brush-border membrane with regard to passive permeation ability, but it also contains a different set of transporter proteins (Masereeuw & Russel 2001; Shitara et al 2005). Efflux proteins in the basolateral membrane include OAT1 and 3 (bi-directional), OCT2 and OAT4C1 (Shitara et al 2005; TP-search transport database). Brush-border membrane transporter proteins involved in secretion include MDR1, OCTN1 and 2 (bi-directional), MRP2 and 4, and OAT4 (bi-directional) (Shitara et al 2005). The secretion capacity of such transporters can be very high. For example, para-aminohippuric acid has a renal extraction ratio close to unity (Rowland & Tozer 1995), and many hydrophilic drugs have CL_R values in the range 200–400 mL min^{-1} (see below).

The fraction tubular reabsorption (f_{reabs}) is determined by the brush-border P_e and surface area, and intraluminal radius, flow characteristics and residence time (Rowland & Tozer 1995). P_e is determined by the characteristics of the brush-border membrane and the compound in question, and by the expression and activity of transporter proteins. It may also depend on drug concentration and pH. There are regional differences in these determinants but these are not well characterised or understood. A concentration gradient is created between urine and blood when filtered water is reabsorbed in the proximal tubuli (80–90% water reuptake), distal tubuli and collecting tubules (Rowland & Tozer 1989; 1995). This gradient, which is also created by the binding of compounds to blood components, could be of several magnitudes. Together with the high passive P_e of the brush-border membrane (compared with the basolateral membrane), this gradient creates a driving force for net diffusion of compounds from the lumen into the blood. The net diffusion of weak acids and bases could be influenced by the pH gradient

between urine (average pH 6.3) and blood (average pH 7.4) (Rowland & Tozer 1995). The pH of urine varies between species (slightly acidic in humans and rodents; neutral in dogs; alkaline in rabbits), as do the types and expression of drug transporters (Walton et al 2004; Shitara et al 2005; TP-search transport database). Thus, there are likely to be species differences in passive and active tubular re-absorption. Transport proteins in the human brush-border membrane capable of reabsorbing compounds are OCTN1 and 2 (bi-directional), OAT4 (bi-directional), PEPT1 and 2 and URAT1 (Shitara et al 2005).

As with tubular secretion and re-absorption, prediction of the extent and fraction of tubular metabolism is complicated, and the potential for metabolism during transport in either direction makes it even more difficult. A comparison with hepatic metabolism capacity could, however, prove valuable for estimating the contribution by the kidneys. The kidneys and liver differ with regard to P_e, residence time, tissue mass, types and distribution of cells, and enzyme levels and activities. MDCK cells appear magnitudes less permeable than the highly permeable hepatocytes (Alpini et al 1986). Data for atenolol can serve as an example of the differences in uptake capacity between kidneys and the liver. The f_u of atenolol is completely absorbed (mainly passively) during the short residence time in the rat liver (~10 s) (Chiou 1983) but it is not reabsorbed to any extent from human renal tubuli (despite a longer residence time) (FASS website). The tubular residence time on the blood side (approximately 5 s) is only about one-quarter of that in the liver, which further indicates a comparably low uptake capacity of the kidneys. Thus, the kidneys are not expected to contribute significantly to the total metabolic CL of compounds with low P_e; a high passive and/or active P_e is required. The kidneys and liver have similar blood flow and microsome protein yield, but the mass of the kidneys is only 15–20% that of the liver (Soars et al 2002; Walton et al 2004). While hepatocytes are known to occupy 78% of the volume and 60% of the cell number in the liver (Meijer & van der Sluis 1989; Roberts et al 2002), the amount and fraction of metabolically active tubular cells in the kidneys is unknown (which further complicates the prediction of CL_R). The amount and fraction appear lower than for hepatocytes. The largest concentrations of renal cytochrome P450 enzymes (CYP450) are found in the proximal tubuli, and renal CYP450 metabolism is reported to take place predominantly at this site (Masereeuw & Russel 2001). Thus, there is a potential to overpredict metabolic CL_R when assuming that the weight of fully active tubular cells equals the total weight of the kidneys. De Waziers et al (1990) investigated the organ distribution of CYP450s 1A2, 2C8–10, 2D6, 2E1 and 3A4 and epoxide hydrolase in man, and found that the concentrations of these enzymes in the kidneys were negligible compared with the liver. Similar results were obtained with rat tissue (De Waziers et al 1990). The in-vitro intrinsic clearance (CL_{int}) of diazepam, a substrate of CYP2C19 and CYP3A4, was 9-times higher in rat liver microsomes than in kidney microsomes, and it was estimated (using a PB IVIV prediction approach with the well-stirred extraction model) that the metabolic CL_R of this drug was only one-fortieth of CL_H (Igari et al 1984). Further support for a minor contribution of the kidneys to the metabolism of CYP450 substrates is provided by data presented by Vickers et al (1993). They found that slices of human liver, but not kidney, were able to metabolise a CYP3A4 substrate. In contrast, kidneys have relatively high expression of UDP-glucuronyltransferases (UGTs) and high glucuronidation CL_{int}. Sutherland et al (1993) showed that phenol UGT HP1 is expressed at similar but relatively low levels in human kidneys and liver, whereas phenol UGT HP4 is more highly expressed in the kidney. They also found similar UGT activities in liver and kidney microsomes for four compounds. Raoof et al (1996) and Shipkova et al (2001) found that in-vitro CL_{int} for glucuronidated compounds (mycophenolic acid and propofol) was higher with human kidney microsomes than liver microsomes, and Soars et al (2001; 2002) demonstrated that in-vitro CL_{int} values from kidney microsomes were 5-fold higher (median; range 0.32–150) than corresponding estimates for the liver. This team also showed that in-vitro glucuronidation CL_{int} by kidney microsomes is considerably lower in dogs than in humans. Proof of the importance of renal metabolism in-vivo is not yet available. Propofol and morphine (moderate passive P_e; MDR1 substrates (Peréz et al 2004; TP-search transport database)) have a CL that exceeds hepatic blood flow (Q_H), suggesting that extrahepatic metabolism (including renal metabolism) may be important for directly glucuronidated substances (Mazoit et al 1990; Raoff et al 1996). As mentioned above, prerequisites for significant renal glucuronidation are probably sufficiently high passive and/or active P_e. The CL_R of morphine approximates CL_{filtr}, which indicates that it is not reabsorbed from the renal tubuli to any significant extent (Goodman Gilman 2001). The possibility that it is actively secreted (by MDR-1) and reabsorbed to the same extent cannot be excluded, however.

Evaluation of methods for predicting renal clearance

Allometry for prediction of renal excretion clearance

In some cases, such as for creatinine and fluconazole, allometry has worked well for prediction of the excretion CL_R in humans (Dedrick 1973; Jezequel 1994). The allometric exponents for these compounds – 0.69 for creatinine and 0.67 for fluconazole – are close to that for GFR (~0.8; Campbell 1994).

Jezequel (1994) found that about 80% of filtrated fluconazole was reabsorbed in the tubuli in seven species, including man. Fluconazole (polar surface area 78 $Å^2$, $f_{u,pl}$ 0.88) is moderately lipophilic and has high intestinal P_e and fraction absorbed (f_a) (0.95) (Zhao et al 2001; Willmann et al 2004). The compound is also a substrate for MDR1 (TP-search transport database), a brush-border transporter involved in tubular drug secretion. Although is possible that secretion occurs and differs between species, the result indicates that secretion of fluconazole is low, reabsorption is mainly passive, and passive reabsorption capacity is similar across species. Animal and human f_{reabs} data for compounds that could potentially show species differences in reabsorption, such as weak acids and bases and actively reabsorbed compounds, have not been reported.

Creatinine has moderate P_e (Fagerholm et al 1999), is eliminated mainly via glomerular filtration, and undergoes minor tubular secretion (creatinine CL is about 20% higher than the GFR; Tattersall 2003) and reabsorption (Wikipedia). This acidic substance has a pK_a of 5 and it contributes to urine acidity (McNamara & Worthley 2001). Acidification of urine to pH below 5 leads to a marked increase in the unionised fraction of creatinine. The importance of degree of ionisation for permeation has been demonstrated by Palm et al (1999). They showed in Caco-2 experiments that the unionised forms of alfentanil (high P_e weak base) and cimetidine (low P_e weak base) were 150- and 30-fold more permeable than the corresponding ionised forms, respectively. Increased tubular reabsorption is therefore expected for creatinine at low tubular/urine pH. On the basis of these data, creatinine and other weak acids with potential for tubular reabsorption are not the most suitable markers for GFR.

Allometry performs more poorly when active processes are involved and efficient. Mahmood (1998) used this approach to predict human CL_R for 10 renally excreted compounds (all actively transported compounds) from animal data (where data from at least three animal species were available) and found that the prediction errors ranged from –85% to +72%. An empirical correction factor for species differences in GFR, Q_R and kidney weight was included but species differences in $f_{u,bl}$, $f_{u,pl}$ and active transport were not measured or taken into consideration. Allometry also performed poorly for napsagatran (Lavé et al 1999b) and talsaclidine (Leusch et al 2000). Leusch et al (2000) used allometry to predict the total CL of talsaclidine, a high CL compound with low degree of plasma protein binding (< 7%) and that is mainly excreted renally (at least 86% excreted unchanged in urine). An allometric exponent of 0.62 was established, and human CL was underpredicted by a factor of about 2. CL in the rat was 2-fold higher than the predicted value, and was about 2-fold higher in male rats than in female rats. The findings demonstrate that the compound undergoes tubular secretion that is different between species, and between sexes in rats. Walton et al (2004) compared the CL of 22 compounds (mostly antibiotics) that are mainly excreted unchanged in the urine (many of which undergo tubular secretion) in several species, and found that CL (in mL min^{-1} kg^{-1}) in mice, rats, rabbits and dogs was, on average, 12-fold (range 2.8–25), 5-fold (1.5–19), 3-fold (0.64–12) and 2-fold (0.26–6.8) higher than in humans. The data show an allometric relationship for average CL data of renally excreted drugs, but there are significant deviations from such a relationship. This was also the case for the animal CL_R data available for some of the compounds. For eight compounds (36%) it was shown that – in contrast to the allometric principle – CL values per kg body weight were higher in larger species than in smaller species.

Relationships between the excretion CL_R and fraction excreted unchanged in urine following intravenous dosing (f_e) in humans and animals are poor. CL_R in humans compared with rats (n = 15; collected from Sawada et al 1985) and humans compared with monkeys (n = 13; data calculated from total and non-renal CL data presented by Chiou & Buehler 2002) showed virtually no relationships for compounds with apparent renal excretion (data not shown). Species differences in CL_R (mL min^{-1} kg^{-1}) reached a maximum of about 20-fold. Sixty percent (humans vs rats) and 15% (humans vs monkeys) of compounds in these data sets had zero CL_R in both species. The differences (at least between rats and humans) do not appear to be related to species differences in f_u. Human and animal (mainly rat) f_e data for 25 compounds were estimated from renal and total CL data available in the literature (Harrison & Gibaldi 1976; Sawada et al 1985; Adedoyin et al 1987; Somogyi et al 1989; Belpaire et al 1993; Dixon et al 1993; Eddershaw et al 1996; Goodman Gilman 2001; Kim & Lee 2001; Cerrutti et al 2002; Chiou & Beuhler 2002; Kato et al 2004). Eleven (44%) of these compounds had an f_e of zero in both humans and rats. Many of these are highly permeable (high tubular reabsorption potential) and unstable compounds (comparably high CL_H). One compound with significant f_e in humans (0.35) had zero CL_R in rats. For 9 of 14 compounds (64%) with apparent renal excretion in both rats and humans (they also have incomplete intestinal f_a and limited passive P_e), the major route of elimination differed between these species. Examples are digoxin (f_e in man = 0.60; f_e in rat = 0.17), quinidine (0.18 vs 0.72) and ranitidine (0.69 vs 0.30). The relationship between human and rat f_e is demonstrated in Figure 1. A similar observation was found for humans compared with monkeys (n = 13; with two exceptions, which were compounds with low/very low f_e; four compounds were renally excreted in one of the species but not in the other) (also shown in Figure 1), and the f_e in dogs in general differed from that in humans. These results demonstrate that the major route of elimination of drugs is not necessarily the same in humans and animals, that highly permeable compounds are most likely to be extensively

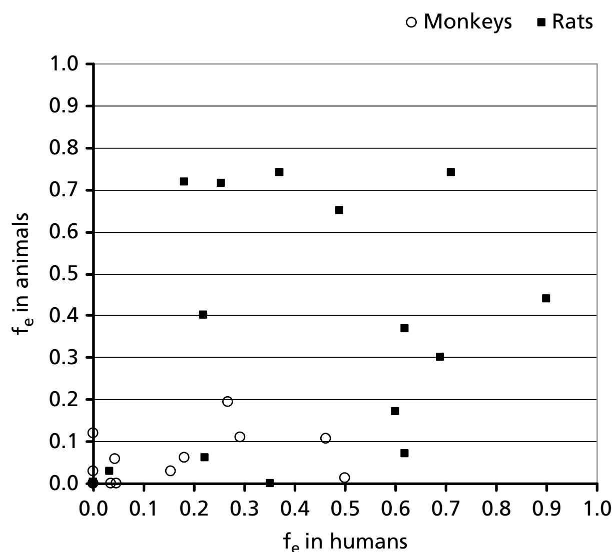

Figure 1 Relationship between fraction excreted unchanged in urine (f_e) in humans vs rats (n = 25 compounds) and humans vs monkeys (n = 13). Zero f_e was observed in both species for 15% of human vs monkey data and in 44% of human vs rat data. (Data were taken from Harrison & Gibaldi 1976; Sawada et al 1985; Adedoyin et al 1987; Somogyi et al 1989; Belpaire et al 1993; Dixon et al 1993; Eddershaw et al 1996; Goodman Gilman 2001; Kim & Lee 2001; Cerrutti et al 2002; Chiou & Beuhler 2002; Kato et al 2004.)

metabolised in all species, and that renal drug excretion varies considerably between species.

Physiologically based prediction of renal excretion clearance

PB IVIV methodology could be applied to the prediction of CL_R (as for CL_H and V_D) although no such attempts have been made, and such an approach is associated with some difficulties (see above). CL_{filtr} could easily be estimated from GFR and $f_{u,pl}$ data, but scaling up from in-vitro active transport to in-vivo requires that in-vitro–in-vivo relationships are established, that an appropriate active transport model is available and that human in-vivo transport data are available. Furthermore, the f_{reabs} must be predictable. Prediction of f_{reabs} requires that f_{reabs} data are available or can be calculated. Uptake capacities across endothelia and epithelia are commonly predicted on the basis of P_e vs fractional (re)absorption relationships.

On the basis of PK data in the literature, it was possible to estimate the in-vivo renal secretion CL (CL_{secr}) and intrinsic CL_{secr} ($CL_{secr,int}$) for a few substances. These four hydrophilic compounds with active tubular secretion, low/intermediate gastrointestinal (GI) P_e and f_a, minor metabolism, and with no apparent tubular reabsorption are famotidine (plasma $CL_R = 330\,mL\,min^{-1}$; $f_{u,pl} = 0.80–0.85$; GI $f_a = 0.38$; OAT3 substrate), ranitidine ($CL_R = 430\,mL\,min^{-1}$; $f_{u,pl} = 0.85$; $f_{u,bl} = 0.77$; GI $f_a = 0.57$; MDR1 substrate), amiloride ($CL_R = 320\,mL\,min^{-1}$; $f_{u,pl} = 0.60$; GI $f_a = 0.50$) and cimetidine ($CL_R = 410\,mL\,min^{-1}$; $f_{u,pl} = 0.80$; $f_{u,bl} = 0.90$; GI $f_a = 0.85$; MDR1, OAT1 and 3, OCT1, 2 and 3 and OCTN2 substrate) (Lin 1995; Mahmood 1997; Irvine et al 1999; Goodman Gilman 2001; Karyekar et al 2004; Peréz et al 2004; Willmann et al 2004; Riley et al 2005; FASS website; TP-search transport database). By assuming negligible tubular reabsorption (as for other hydrophilic compounds with similar GI f_a; see below) and metabolism, the in-vivo plasma $CL_{secr,int}$ values of these drugs were estimated to about 420, 740, 640 and 720 $mL\,min^{-1}$, respectively. These estimates are based on renal plasma flow rate. Based on blood flow, in-vivo blood $CL_{secr,int}$ for ranitidine and cimetidine were estimated to be approximately 810 and 640 $mL\,min^{-1}$, respectively, and tubular extraction ratios were estimated to be 0.34 and 0.32, respectively. Since some of these compounds are substrates for transporters capable of reabsorbing compounds, it cannot be ruled out that $CL_{secr,int}$ values have been underestimated to some extent. Data for these compounds could be useful as references for establishing in-vitro/in-vivo relationships for active tubular secretion.

A general rule has been that the tubular reabsorption will be complete or near complete for compounds with a log $D_{7.4}$ (log D at pH 7.4) above 0 (Smith et al 1996). A cut-off value of this magnitude is, however, questionable because such a cut-off log $D_{7.4}$ is not apparent for uptake from other organs. For example, in the intestines (Artursson et al 2001), active processes that are less dependent on lipophilicity could be involved. The urine pH is lower than 7.4 (6.3), and the uptake capacity generally correlates better with P_e than with lipophilicity (Rowland & Tozer 1995; Yazdanian et al 1998; Stenberg et al 2002). High CL_R values have been found for compounds with log $D_{7.4}$ up to 2. Van de Waterbeemd and Jones (2003) have compared human CL_R and log $D_{7.4}$ for beta-blockers. The highest observed CL_R for substances with log $D_{7.4}$ of −0.5–0.5, 0.5–1, 1–1.5 and 1.5–2.0 were approximately 340, 140, 90 and 100 $mL\,min^{-1}$, respectively. In a report by Smith et al (1996), the CL_R in man for 30 compounds (beta-blockers and highly protein-bound non-steroidal anti-inflammatory drugs) appeared to decrease in a linear manner with increasing log $D_{7.4}$, and the highest observed CL_R for compounds with log $D_{7.4}$ of 0–0.5, 0.5–1 and 1–1.5 were approximately 240, 40 and 1 $mL\,min^{-1}$, respectively.

Potential for high tubular reabsorption is not the only reason for low CL_R of lipophilic compounds. Such compounds are also more likely to be extensively bound to blood components and to have low CL_{filtr} (Tillement et al 1984; Meijer & van der Sluijs 1989). Evaluations of tubular reabsorption have not taken CL_{filtr} into consideration.

PK data of 29 compounds in Goodman Gilman (2001) were used to approximate the f_{reabs} in humans in-vivo. These f_{reabs} data (ranging between approximately 0 and 1) were correlated to passive in-vitro P_e (immobilised lipid bilayer P_e at pH 7.4; taken from Willmann et al 2004). The relationship estimated between passive in-vitro P_e and average f_{reabs} for compounds with (upper limit for secreted compounds) and without apparent active secretion are presented in Figure 2, together with similar relationships for intestinal and hepatic f_a (uptake for the unbound fraction during one passage through the organs). Intestinal and liver data were taken from a recent study by Fagerholm (2007d), which presented a P_e-based classification system (PCS) for prediction of PK, elimination routes and drug–drug interactions. The uptake capacity of the human brain is also included. The data show that f_{reabs} is negligible for compounds with passive P_e less than or equal to

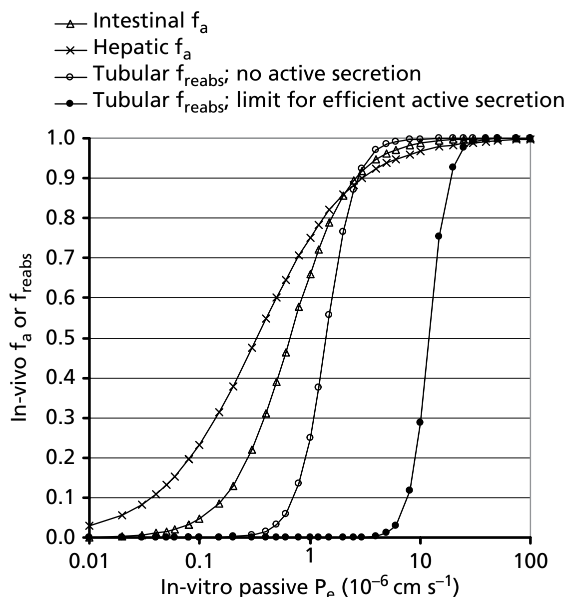

Figure 2 Relationship between in-vitro passive permeability (P_e; obtained with immobilised lipid bilayers at pH 7.4) and tubular fraction reabsorbed (f_{reabs}) and intestinal and hepatic fraction absorbed (f_a) in humans in-vivo. Data were taken from Fagerholm (2007d).

that of atenolol, and that f_{reabs} is complete or near complete for substances with passive P_e equal to or above that of carbamazepine. In this P_e model, carbamazepine has 34-fold higher passive P_e than atenolol. For compounds with intermediate P_e the relationship is irregular and f_{reabs} is difficult to predict. Figure 3 shows the relationship between passive in-vitro P_e and in-vivo f_e for 23 of these compounds. Thus, renal excretion is not anticipated to be the major route of elimination for compounds with P_e similar to or greater than that of carbamazepine, and tubular reabsorption is not expected for low-P_e compounds. The results demonstrate the difficulty of predicting f_{reabs}, f_e and CL_R for actively secreted compounds with low and intermediate passive P_e.

The following equations could be useful for prediction of excretion CL_R.

If there is no active transport and passive P_e is less than or equal to that of atenolol:

$$CL_R \approx GFR \times f_{u,pl}.$$

If there is no active transport:

$$CL_R \approx (GFR \times f_{u,pl}) \times (1 - f_{reabs*}); \text{ *See Fagerholm (2007d).}$$

If the passive P_e is higher than or equal to that of carbamazepine: CL_R 0.

If there is active secretion and passive P_e is less than that of carbamazepine:

$$CL_R \approx [(GFR \times f_{u,pl}) + (CL_{secr*} \times f_{u,bl} \times Q_R) / (CL_{secr*} \times f_{u,bl} + Q_R)] \times (1 - f_{reabs**})$$

*Scaled up from in-vitro to in-vivo. **See Fagerholm (2007d).

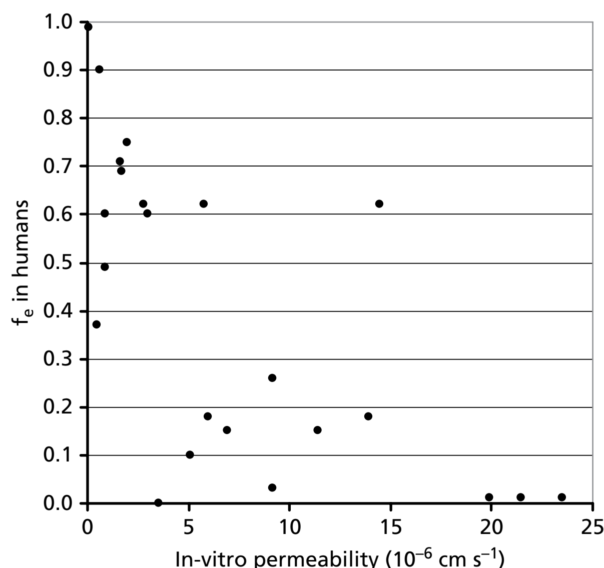

Figure 3 Relationship between in-vitro passive permeability (P_e; obtained with immobilised lipid bilayers at pH 7.4) and fraction excreted unchanged in urine (f_e) in humans in-vivo (n = 23). Data were taken from Goodman Gilman (2001) and Willmann et al (2004).

I have previously shown that compounds with moderate and high passive P_e generally have a CL_R below 150 ml min^{-1}, and that compounds with low passive P_e could have a CL_R exceeding this limit (Fagerholm 2007d). Thus, by developing CDs with some metabolic degradation and moderate/high passive P_e, renal excretion could be minimised or avoided.

Tubular metabolic clearance

The amount and fraction of tubular cells with UGT activity, and regional P_e and UGT activities are unknown, and tubular cell uptake is difficult to estimate. Thus it is also difficult to predict metabolic CL_R.

Soars et al (2001, 2002) estimated in-vitro CL_{int} data for eight glucuronidated (by UGTs) compounds from human kidney microsomes and used these data to predict the in-vivo CL_R. A PB IVIV approach with the well-stirred model was used to predict in-vivo CL_{int} from in-vitro CL_{int} data, and the number of cells and amount of microsomal protein in the kidneys. They found that kidney CL_{int} values were 5-fold (range 0.32–150) higher than corresponding estimates for the liver, and predicted renal and hepatic metabolic CL were similar. Based on the well-known low metabolic activity of liver microsomes, this approach has the potential to underestimate in-vitro and in-vivo renal CL_{int}, and CL. Furthermore, the absence of an outer cell membrane that drugs have to permeate could affect predictions for compounds with low P_e.

A simple and crude rule could be to assume that the total metabolic CL of highly permeable (passive and/or active) substances glucuronidated by UGTs is somewhat higher than predicted from hepatocyte CL_{int} data, and that the metabolic CL_R for CYP450 substrates and low-P_e compounds can be neglected. By assuming that metabolically active hepatocytes and proximal tubular cells occupy three-quarters (Meijer & van der Sluis 1989; Roberts et al 2002) and half of the liver and kidney volumes/weights, respectively, and that kidney mass is 15–20% of the liver mass (Soars et al 2002; Walton et al 2004), a highly permeable compound with renal microsome CL_{int} that roughly equals hepatic microsome CL_{int} will have a metabolic CL_R/CL_H ratio between ~0.1 (at low CL_{int}) and ~1 (at very high CL_{int}; $(Q_R + GFR)/Q_H$).

Conclusion

Methods for prediction of the excretion and metabolic CL_R in humans have been evaluated. The few available methods (allometry for excretion CL_R and a PB IVIV method based on microsome data for metabolic CL_R) perform poorly for a number of reasons. Renal drug excretion differs considerably between species, and major routes of elimination of a drug may differ between humans and animals, which also limits the value of animal PK data.

Many compounds are eliminated mainly by renal excretion, and drug–drug transport interactions are common for such substances. On this basis, it is important that CL_R prediction methods are improved and applied.

For several reasons CL_R is difficult to predict. Improvements may be possible by applying a PB IVIV approach, including a method for estimation of in-vitro $CL_{secr,int}$ and

establishment of the in-vitro/in-vivo relationship for active secretion. The newly developed PCS for prediction of PK, elimination routes and drug–drug interactions could also be useful. The relationship between passive in-vitro P_e and in-vivo f_{reabs} is established in the PCS.

CYP450 substrates are expected to undergo negligible metabolic CL_R. The metabolic CL_R of high-P_e UGT substrates may however contribute to the total CL to some extent.

One way to avoid renal excretion (and poor PK predictions) and drug–drug transport interactions could be develop CDs with sufficiently high hepatic CL_{int} and high passive P_e (good/complete tubular reabsorption; hepatic metabolism as main elimination route).

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